**PATENT APPLICATION**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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|---|---|
| In re Application of: | Docket No: **Q101061** |
| **Masahiro KAJINO et al** | Conf. No.: **7356** |
| Appln. No.: **10/533,833** | Group Art Unit: **1625** |
| Filed: **May 3, 2005** | Examiner: **Marby, John** |

For: **RECEPTOR REGULATOR CONTAINING A NITROGEN-CONTAINING RING DERIVATIVE HAVING AN AMINO GROUP**

**DECLARATION UNDER 37 C.F.R. § 1.132**

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Naoki TARUI, hereby declare and state:

THAT I am a citizen of JAPAN;

THAT I have received the degree of Master of Agriculture in March of 1985, from The Osaka Prefecture University;

THAT I have been employed by Takeda Pharmaceutical Company Limited (the Assignee of the above mentioned patent application) since April of 1985, where I hold a position as Research Manager (job title), with responsibility for research and development of medicine.

1. I am one of the inventors of the above-identified application and am familiar with the subject matter thereof;

2. I have read the Office Action mailed November 24, 2008, and the references cited therein and am familiar with the subject matter thereof; and

3. In order to compare the neuromedin U receptor antagonistic activity of the compound of the reference as cited by the Office with that of the compound claimed in the above-identified application (the compound of the present invention), the same tests as Test Examples 1 to 4 described in the specification of the above-identified application was conducted under my direction and supervision.

NEUROMEDIN U RECEPTOR BINDING INHIBITING

EXPERIMENT OF TEST COMPOUNDS

**Test Compound A** (the compound 8f of Kudzma et al., *J. Med. Chem.*, 32:2534-2542 (1989)):

N-[1-benzyl-4-(4-methylthiazol-2-yl)-4-piperidinyl]-N-phenylpropionamide

**Test Compounds B to E** (the compound of the present invention):

**Test Compound B:**

N-[1-Benzyl-4-(4-methylthiazol-2-yl)-4-piperidinyl]-N-phenylacetamide oxalate (the compound of Reference Example 39 of the present invention)

**Test Compound C:**

N-[1-Benzyl-4-(4-methylthiazol-2-yl)-4-piperidinyl]-N-phenylacetamide fumarate (the compound of Reference Example 101 of the present invention)

**Test Compound D:**

N-[1-Benzyl-4-(4-methylthiazol-2-yl)-4-piperidinyl]-N-(2-methylphenyl)acetamide oxalate (the compound of Reference Example 80B of the present invention)

**Test Compound E:**

N-[1-Benzyl-4-(4-methylthiazol-2-yl)-4-piperidinyl]-N-(3-fluorophenyl)acetamide oxalate (the compound of Reference Example 83B of the present invention)

**Preparation of cell membrane fraction expressing human FM-3**

$1x10^8$ CHO/human FM3 cells were added to 10ml of a homogenizing buffer (50mM Tris hydrochloride buffer, pH 7.5, 5mM EDTA, 0.5 mM PMSF, 0.1 μg/ml PepstatinA, 4 μg/ml E-64, 20 μg/ml Leupeptin) and the mixture was ground using Polytron (12,000 rpm, 15 seconds x three times).

The cell ground mixture was centrifuged (1,000g, 10 minutes ) to obtain a supernatant. Then, the supernatant was ultracentrifuged (Beckman type 30 rotor, 30,000 rpm, 1 hour), and the resulting precipitate was used as a CHO cell fraction expressing human FM3.

**Preparation of cell membrane fraction expressing human TGR-1**

$1x10^8$ CHO/human TGR-1 cells were added to 10ml of a homogenizing buffer (50mM Tris hydrochloride buffer, pH 7.5, 5 mM EDTA, 0.5mM PMSF, 0.1 μg/ml PepstatinA, 4μg/ml E-64, 20 μg/ml Leupeptin), and the mixture was ground using Polytron (12,000 rpm, 15 seconds x three times).

The cell ground mixture was centrifuged (1,000 g, 10 minutes) to obtain a supernatant. Then, the supernatant was ultracentrifuged (Beckman type 30 rotor, 30,000 rpm, 1 hour), the resulting precipitate was used as a CHO cell fraction expressing human TGR-1.

**Preparation of Neuromedin U-8 labeled with isotope**

Neuromedin U-8 labeled with an isotope for using in a binding inhibiting experiment was prepared as follows:

To a solution of 10μl of 100μM Neuromedin U-8 (Buchem) in distilled water was added 0.01mg/ml of lactoperoxygenase (Sigma). After mixing, [$^{125}$I]NaI 37MBq (Amersham Biosciences) was added thereto. Further, 10μl of 0.005% $H_2O_2$ was added thereto and the reaction was performed for 10 minutes. After addition of 600μL of 0.1% TFA, purification was performed by HPLC using TSK gel ODS-80Ts (100mm x 4.6mm I.D, Tosoh Corporation) to obtain labeled Neuromedin U-8.

**Binding inhibiting experiment of test compounds using human FM-3 expressing cell membrane fraction and isotope-labeled Neuromedin U-8**

Human FM-3 expressing CHO cell membrane fraction was diluted with a membrane diluting buffer (50mM Tris hydrochloride buffer, pH 7.5, 5mM EDTA, 0.5mM PMSF, 0.1μg/ml Pepstatin, 20μg/ml Leupeptin, 4μg/ml E-64) to prepare a cell membrane fraction solution for an assay having a protein concentration of 20μg/ml. Each 25μl of a membrane fraction solution for an assay was dispensed in a 96-well microtiter plate, and 25μl of a membrane diluting buffer containing 400pM [$^{125}$I]-labeled Neuromedin U-8 and 50μl of a solution in which dimethyl sulfoxide was diluted 100 volume-fold with a membrane diluting buffer for investigating total binding, 25μl of a membrane diluting buffer containing 400pM [$^{125}$I]-labeled Neuromedin U-8 and 50μl of a 10% dimethyl sulfoxide-containing membrane diluting buffer containing 20μM non-isotope-labeled Neuromedin U-8 for investigating non-specific binding, and 50μl of a solution in which a solution of a test compound in dimethyl sulfoxide was diluted 100 volume-fold with a membrane diluting buffer and 25μl of a membrane diluting buffer containing 400pM [$^{125}$I]-labeled Neuromedin U-8 for investigating binding inhibiting activity of a test compound

were added, respectively, to react them at 25°C for 1.5 hours. The mixture was filtered with a filter plate (GF/C, Whatmann) and the filter was washed with a washing buffer (50 mM Tris hydrochloride buffer, pH 7.5) six times, 20μl of Microscinti 20 (Perkin Elmer Lifescience) was added, and radioactivity was measured with Topcount (Perkin Elmer Lifescience). Specific binding is a value obtained by subtracting non-specific binding from total binding. Human FM-3 binding inhibiting activity of a test compound is indicated by a ratio of a value obtained by subtracting radioactivity of a cell membrane fraction with a test compound added from total binding relative to specific binding.

**Test Results**

Results of human neuromedin U receptor FM-3 binding inhibiting experiment of test compounds are shown below.

| Test Compound | $IC_{50}$ (nM) |
|---|---|
| A | 46 |
| B | 24 |
| C | 22 |
| D | 18 |
| E | 22 |

As described in detail, we compared the neuromedin U receptor antagonistic activities of Applicants' claimed compounds to compound 8f of Kudzma et al. (referred to as Test Compound A) which is the closest compound among the compounds indicated in the cited references (i.e.,

Lin et al. '192, Lin et al. '749 and Lin et al. '120 and Kudzma et al.) to the compound at issue in the rejection. This compound corresponds to the compound of Reference Example 38 described in the patent application specification. We found that the neuromedin U receptor antagonistic activities of the presently claimed compounds to compound 8f of Kudzma et al. were much higher than that of N-[1-benzyl-4-(4-methylthiazol-2-yl)-4-piperidinyl]-N-phenylpropionamide (Compound 8f of Kudzma et al.). The compound 8f (wherein $R^{2b}$ is ethyl) exhibited a neuromedin U receptor binding inhibiting activity and had an $IC_{50}$ of 46 nM. With regard to the neuromedin U receptor binding inhibiting activity, however, the compound wherein $R^{2b}$ was methyl as described in claim 23 had a higher activity than the compound wherein $R^{2b}$ was ethyl. For example, the compound of Reference Example 39, which is the same as the compound 8f except that $R^{2b}$ is changed to methyl, had an $IC_{50}$ of 24 nM (see, Table 1). The compound of Reference Example 101 had an $IC_{50}$ of 22 nM, the compound of Reference Example 80B had an $IC_{50}$ of 18 nM, and the compound of Reference Example 83B had an $IC_{50}$ of 22 nM. The compound having $R^{2b}$ of methyl smaller than ethyl had a higher activity. Such a structure-activity correlation is unexpected. Lin et al. '192, Lin et al. '749 and Lin et al. '120 and Kudzma et al. do not teach or suggest such an unexpected superior result of the present invention.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Feb. 17, 2009

Naoki TARUI